Chapter 9

Infection by Human Papillomavirus (HPV), Chlamydia trachomatis and Ureaplasma urealyticum, in Relation with Reproductive Failure

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Additional information is available at the end of the chapter

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Abstract

Recent studies suggest that besides oncogenic capacity, HPV could have etiological role on infertility, but more evidence is necessary to confirm these results. We present in this chapter the microbiological and clinical outcome of 104 infertile women aleatory selected, from northeast of Mexico: 84.6%, with genital infection (GI) by multiple germs: *Chlamydia trachomatis* (*Ct*) [86.5%], HPV [49%], *Ureaplasma urealyticum* (*Uu*) [47.11%] and *Mycoplasma hominis* [35.57%]. Significant association (P ≤ 0, 05) was observed between the HPV presence and *Uu* diagnosis, assisted-reproduction unsuccessful like previous treatment, cervical cytology with inflammatory process, multiple sexual partners, white-dense-mucous, secretion into the vagina, and HPV diagnosed in early years. The more frequent genotypes of HPV present in the infertile women studied were 6/18/16/58/11 and 68. In 60% of them, more than two genotypes were founded. The most frequent associations of high-risk HPV (HPVhr) were 16/18, 16/58, 16/33, 16/52 and 18/58. Considering the isolate or combined presentation of HPVhr, 79.5% of these women would have a potential to develop cervix carcinoma. GI by HPV/*Uu*/*Ct* affects the fertility. Infertile women with GI that include these microorganisms with probed (HPV/*Ct*) or suspicious carcinogenic effect (*Uu*) would be considered a group of high risk for cervical cancer.

Keywords: genital infection, HPV, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, Infertility, risk for cervical cancer

1. Introduction

At present, reproductive failure affects to a great population. Human infertility is defined by World Health Organization, like the absent of conception and outcome of healthy baby borne, after 1 year of unprotected intercourse. Infertility is a health complex problem with low quality life for the couples. Estimations on 2010 referred 1.9 and 10.5% of primary and secondary infertility, respectively, on women at reproductive age (20–40 years old). A high prevalence of fertility health is documented in South of Asia, Medium East, Central Europe, East Europe, and Central Asia [1].

Genitourinary unspecific infection is associated with unexplained infertility, subfertility, obstetric and gynecologic complications that not have known clear etiology for several decades like recurrent abortion, premature delivery, placenta dysfunction and preeclampsia [2, 3]. As well, assisted reproduction treatment for infertility had been associated with genital infection [4–7]. Specific treatment for genital infection is recommended to improve the successful of *in vitro* fertilization an embryo implantation [8, 9]. The low age of sexual activity initiation and with multiple sexual partners is a recognized risk factor for sexually transmitted genitourinary infection, but the absent or subtle clinical manifestations prevent the opportune treatment and persisting infection for long time and thus may exert detriment on the reproductive function [10].

Besides the still controversial participation of HPV on fertility and obstetric complications, the major bacteria implicated for a long time are *C. trachomatis,* and other atypical bacteria treated in next section: *U. urealyticum*, *M. hominis,* that were considered for a long time an improbable cause of female internal genital infection, were isolated from endometrial tissue and material obtained by hysteroscopy and laparoscopy procedures, in women with tubary obstruction, hidrosalpinx, and adherence syndrome [11–15]. In recent years, another species of Mycoplasmataceae, *M. genitalium*, has been considered an emerging sexually transmitted infection that exerts damage in female genital tract and is implicated in fertility problems [16–18].

2. Human papillomavirus

2.1. Classification

HPV belongs to *Papoviridae* family microorganism and comprises a group of approximately 200 small DNA viruses. According to its tissue tropism, HPV has been classified like *Alpha* HPV that affects epithelial mucosae and is subclassified into high risk and low risk of carcinogenic potential *Beta, gamma, nu* and *mu.* HPV has more potential to affect cutaneous epithelia and more associated with papilloma and external warts and had been associated with no melanocytic skin carcinoma [19, 20].

2.2. Epidemiology

The HPV infection has an epidemiologic importance. Registered prevalence is influenced by diagnostic test applied, the number and age of individuals of studied population, the

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geographic region studied [21]. Nevertheless, actually are considered the most common sexually transmitted viral infection worldwide, present in 11–12% of population and are 14 million people infected by first time each year. The incidence of HPV infection in the United States is one of higher, as 44.8%, and is present principally in women 20–24 years old [22]. In Mexico from 2005 to 2010, urogenital candidiasis and HPV infection present an incidence of 12.3/100,000 habitant between 15 and 24 years old and were higher between 20 and 24 years old. In this country had been estimated that toward 2050, there are not a clear tendency to diminish of the HPV infection. This situation is very worrying due to the potential malignancy of the lesions [23]. On the records of National Institute of Statistic and Geography of Mexico, were informed that 4417 women died by cervical cancer, in 2013 [24].

2.3. Biomedical importance

HPV infect principally undifferentiated keratinocytes into de basal level of stratified squamous epithelia, from mucous genital and oropharyngeal epithelia cutaneous and, as well as glandular cells of endocérvix [25–27]. HPV had been recognized as definitive anogenital carcinogen for male and female, mainly in uterine cervix cancer [28], alone or in combination with other germs [29]. Some estimations show that the presence of HPV represents 12 more opportunities to develop cervical cancer than general population [30] and the HPV infection the most important between the factor risk for cervical cancer [21]. Recently, HPV was associated, lung cancer [31–34] to larynx and pharynx carcinoma that their incidence are increasing in the last years [35, 36]. The global incidence of head and neck squamous cells and cervical cancer is similar; infection at both sites is strongly associated with sexual behavior: similarities in chromosomal aberrations, gene expression, and methylation and micro RNA profiles between Positive HPV head and neck squamous cells and cervical cancer. All of these observations were referred as argue to carry out comparative epidemiologic study of HPV infection and associated with carcinoma of head and neck and cervical cancer [37].

2.4. Their role in reproductive failure

Although is well known than many authors are interested to search carcinogenic roll of HPV, there are only a few studies about the effect of HPV infection on human reproduction [38–41]. There is evidence showing the adherence of HPV to the equatorial segment of sperm cell [42]. Epidemiologic data about the infection by HPV in infertile men associated high levels of seminal leukocytes, with altered movement and morphology of head sperm, with the HPV infection [43–45]. Previously, experimental studies had been demonstrated chromosomic damage in HPV infected sperm, depending of genotypes 16 and 3 [46, 47]. An association has been found between cervical HPV of high risk and premature membrane rupture and preterm new borne [48–50]. The genital infection by more than one genotype of HPV was higher in recurrent early lost pregnancy, and HPV was identified in placenta tissue of preterm delivery by preeclampsia [41]. The frequency of cervical HPV infection and high-grade lesion was higher in women that have indicated assisted reproduction than general population [6] and successful of assisted reproduction were affected by the presence of HPV [5, 7]. The HPV

transmission from mother to child *in utero* was informed in several investigations [51–54]. These authors remark the risk to appear papillary lesions in oropharynx and larynx of new born from mothers infected by HPV.

3. Chlamydia trachomatis

3.1. Biology and classification

The family Chlamydiaceae consists of two clinically important genera, *Chlamydia* and *Chlamydophila*, with three species responsible for human disease: *Chlamydia trachomatis*, *Chlamydophila psittaci*, and *Chlamydophila pneumoniae* [55]. *C. trachomatis*, as all the members of the family Chlamydiaceae, is an obligate intracellular parasite whose developmental cycle occurs within a eukaryotic host. Infection of eukaryotic host cells is initiated by the metabolically inactive and the infectious elementary bodies (EBs). Through largely unknown mechanisms, EBs attach to and induce their internalization by host cells. Within the first few hours post infection, EBs differentiate to the larger and more pleomorphic reticulate bodies (RBs), which are metabolically active, noninfectious, and replicative. At the end of a successful developmental cycle, the cell lyses, releasing the EBs [56, 57].

3.2. Medical relevance

Chlamydia trachomatis is the most prevalent sexually transmitted bacterial infection, recognized worldwide, which causes a wide spectrum of diseases, including salpingitis, endometritis, and uterine, cervical lesions with scarring process, which often causes infertility in women [58]. Serotypes D-K of *C. trachomatis* has an etiologic relation to pelvic inflammatory disease; it has been isolated from superior genital tract in a quarter of women with pelvic inflammation and is possible that chronic process would be in relation to ovarian cancer [59]. The role of *C. trachomatis* with regard to inducing male factor infertility is a matter of debate. Chlamydial infection could potentially exert a strong influence on male infertility, as it is the main cause of urethritis and accessory gland inflammation in men. Sequelae of ascending infections might be occlusions in the canaliculi system of the genital tract, damage of the epithelial cells involved in spermatogenesis, and immunoreaction with the production of antisperm antibodies [60, 11]. Also, the relationship of *C. trachomatis* infection with semen quality and sperm morphology is still controversial [61, 62]. In men, are documented that *Chlamydia trachomatis* is responsible of epididymitis, orchitis, prostatitis and urethritis [63–66]. Then more, there are many evidence of that this infection cause sperm damage with low motility, altered morphology, and diminish of sperm concentration, with detriment of male fertility [67–71]. Sexually transmitted infections are hypothesized to play a role in the development of prostate cancer, perhaps due to inflammation-induced oncogenesis [71]. Eventually, more evidence supports this affirmation. An investigation to evaluate the possible role of *Chlamydia trachomatis* in the pathogenesis of prostate cancer assessed the presence of this bacterium in prostate biopsies of patients with prostate cancer, patients with benign prostatic hyperplasia

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(BPH) and control subjects. Tissue sections were analyzed using a direct fluorescent-antibody (DFA) assay. When proliferative areas were compared, *C. trachomatis* was most frequently detected in prostate cancer group that in BPH patients ($p = 0.006$). In inflammation areas, *C. trachomatis* was most frequently detected in prostate cancer patients that in control subjects ($p = 0.008$). These data suggest an association between the presence of *C. trachomatis* and prostate cancer [72].

4. Mycoplasmataceae Genus

4.1. Biology and classification

Mycoplasma are microorganisms derived from Gram-positive bacteria characterized by: streamlined and very small genome, the absence of a cell wall, requiring cholesterol for membrane function and growth, and displaying genetic economy that determine a strict dependence of the host for nutrients and refuge [73, 74]. They have a parasitic lifestyle, invading target cells and existing and replicating for extended periods intracellularly [75]. From 200 species established into class Mollicutes, six of them: *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Mycoplasma primatum*, *Mycoplasma spermatophilum* and *Mycoplasma penetrans* have like their main site of colonization, the genital tract [76]. These microorganisms can be commensal in low genitourinary tract of sexually active men and women and can live in close relationship to mammalian cells. Nevertheless, they may cause disorders and pathologies associated with infertility: non-gonococcal and non-chlamydial, epididymitis, urolitiasis, as well with reproductive problems and infertility [77–84].

4.2. Medical relevance

Gnarpe and Friberg in early seventies informed for the first time a high percentage (85%) of infertile patients with *Ureaplasma urealyticum* isolated from semen by specific culture, compared to low percentage (22%) in fertile men. Then, these authors showed by scanning electron microscopy, Mycoplasma adhered to sperm tail [85]. Similar observation was done in the following years by different authors [86–90]. The use of PCR techniques confirmed the presence of *Ureaplasma urealyticum* in seminal samples of subfertile men with sperm alterations [62, 91, 92]. Since two before decade, had been affirm that Mycoplasma have carcinogenic potential due to the long latency an infection chronicity that may induce malignant cellular transformation [93]. Recently, experimental studies demonstrated induction of genic expression altered in prostatic and cervical cells and progression to malignant transformation [94, 95]. *Mycoplasma hominis* and *Mycoplasma hyorhinis*, a mycoplasma frequently present in patients with AIDS, induce malignant transformation with increased karyotypic entropy, chromosomal aberrations and polysomy, in BPH-1 cells, after 9 months that were infected with these germs [96]. These observation had been supported a relationship between prostatitis and cancer, in etiologic association referred in 2002 [97].

5. Mechanism of genital coinfection by HPV, *chlamydia trachomatis* and *mycoplasmas*

The cervical overlaying columnar epithelia can be invaded by *Ureaplasma urealyticum* and *Chlamydia trachomatis* that produce severe inflammatory response, with discharge of exudative secretions and clinical signs and symptoms [98, 99]. Nevertheless, usually the appropriated diagnostic test will not be applied, and the treatment generally is unspecific. The presence of inflammatory cells in the genital ducts enhances the adherence of virus [100, 101], and *Chlamydia trachomatis* has been considered an enhancer to the entry of human immunodeficiency virus and HPV, the only one germ directly associated with the etiology of cervical cancer [101–104]. Several studies have demonstrated an association between *Chlamydia trachomatis* and a high risk HPV persistence [105, 106]. A study realized by Paba et al., (2008), in patients with intraepithelial neoplasia or cervical cancer, confirmed the association of *Chlamydia trachomatis* and multiple high risk HPV persistence, and like a consequence of viral integration, inhibition of cell apoptosis, overexpression of E6/E7 oncogenes and cellular transformation [107].

6. Our experience

6.1. Studies on male infectious factor of infertility

Our group has reported different studies in couples with infertility of unknown etiology, from northeast Mexico. In that patients with spontaneous and recurrent abortion and failure in assisted reproduction treatments, was frequently detected the presence in seminal fluid, urethral swab and cervix and vagina secretions, of *Chlamydia trachomatis* and *Ureaplasma urealyticum,* and *Mycoplasma hominis*. The diagnosis of *Chlamydia trachomatis* was made by direct immunofluorescence, the gold standard for this microorganism for a long of time. For detection of *Ureaplasma urealyticum* and *Mycoplasma sp.,* we practiced specific media culture, considered too, the gold standard. In morphologic seminal analysis, we described the presence of elementary bodies and inclusion vacuoles of Chlamydia and bacterial particles similar to Mycoplasmas, inside the cytoplasm, adhered to the principal piece and into the middle piece of the sperm [108].

A pattern of structural sperm alteration and inflammatory reaction with sperm phagocytosis, by leucocytes and macrophages, were described by semi thin section at light microscopy and thin section by transmission electron microscopy [109–113]. Beside this the infectious process result a diminish motility, vitality, linear movement and sperm concentration. These seminal parameters were associated with high levels of sperm chromatin fragmentation (SCD test), and high levels of reactive oxygen species (ROS) detected by NBT test. The antibiotic, antioxidant and drugs against the inflammation treatment determine the reduction of bacteriospermia, increase percentage of normal sperm, especially from acrosome damage, middle piece flagellum and nuclear defects. After 6 weeks of treatment, the probability of outcome of pregnancy with healthy newborn increased, and seminal parameters of predictive value were chromatin fragmentation, bacteriospermia and head sperm anomalies [114–117].

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6.2. Studies on female infectious factor of infertility

After confirming that a very high percentage of couples in northern Mexico with incapacity to procreate, have *Chlamydia trachomatis* and *Ureaplasma urealyticum* infections in the ducts and organs of the seminal tract, associated with detrimental damage of fertilization capacity of the sperm, we studied the endometrium histopathology and *endometrialis bacteria.* Although endometritis is included into the internal genital infection, present in a high percentage of infertile couples, the study of endometrial biopsy is out of the diagnostic routine evaluation of infertility female factor. Microscopic analysis was performed on paraffin embedded and H&E stained tissue sections, observed at 1000X. In all the cases, it was possible to recognize, the presence of bacterial particles, identified according to their morphology, such as Chlamydia or Ureaplasma, which were previously diagnosed in genital secretions. The most frequent histopathologic findings at the endometrial tissue were interstitial edema (93%), lymphocytic and polymorphonuclear subepithelial infiltrate (72%) and in 48%, plasmocytic subepithelial infiltrate. The cell denudation of the lining epithelia was observed in 69% of biopsies, and in 62% of them, intracytoplasmic vacuole of epithelial cells, known as spongiosis, was observed. In a low percentage of cases, intraepithelial inflammatory cells (lymphocytes and polymorphonuclears) were found at the endometrium, both lining and glandular epithelia. Germinal center of inflammatory cells in the connective stroma also was observed. The concluding results of this study showed the invading endometrium with *Chlamydia trachomatis* and *Ureaplasma urealyticum* that were present too, into the mucus of uterine cavity and cervical duct. In view of glandular and stromal changes, inflammatory reaction and presence of bacteria into the stromal tissue and glandular and lining epithelia, endometritis due to *Chlamydia trachomatis* and *Ureaplasma urealyticum*, should be considered like an adverse condition to the female fertility as well implantation and normal embryo development. The clinician must consider the study of endometrial biopsy in all patients that need evaluation for infertility, even more if signs and symptoms of internal genital infection or chronic inflammatory process are present. Early diagnosis of genital infection and laboratory test performance looking for *Chlamydia trachomatis* and *Ureaplasma urealyticum* and its corresponding treatment could be prevent reproductive failure and scarring injuries and maybe can improve the results of reproduction treatments [118, 119]. While male genital infection by *Chlamydia trachomatis and Ureaplasma urealyticum* is generally asymptomatic, the women have a variable signs and symptoms of the sick, and clinical improvement is easier to appreciate; in male, their low and subtle clinical signs of infection and the treatment results can be see only by the study of seminal parameters in relation to the infection. For some years, we have focused on the investigation of the clinical and pathogenic aspects of the internal genital infections as well as the integral therapeutic management of male and female reproductive pathologies named as treatment of "binomials gineco-andrologic" of the infectious factor of Infertility [120, 121].

6.3. Study of HPV in infertile couples

In addition of sexually transmission of *Chlamydia trachomatis and Ureaplasma urealyticum*, along the clinical evaluation, is necessary to be in alert to another sexually transmitted germ. This chapter focuses on the results of the first work that we carried out about the importance

of the presence of HPV in the population with infectious factor of infertility. The aim of this analysis was to establish the HPV and genotype prevalence, the relationship of HPV presence with other microorganism, and to describe clinical findings of the studied group of patients.

6.3.1. Patients, material and methods

6.3.1.1. Study population

For the present analysis, samples of mucous secretion from cervix and vagina and endocervical scrapes of 104 women randomly selected women, from patients attended at private clinic in Monterrey, México, for infertility and genitourinary infection since 2003–2014. Patients were recruited by verbal invitation at the time of consultation. They gave voluntary authorization, by Informed consent, to use the clinical data and results of the laboratory test for statistical analysis. Ethical issues were in accordance with the Helsinki Declaration and endorsed by the Ethics Committee of our institution (PA15-001). All selected patients had polymerase chain reaction (PCR) test for HPV in genital secretions and were tested by direct specimen test Kit for *Chlamydia trachomatis* detection (MicroTrak; Trinity Biotech, Wicklow, Ireland); the isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis* was done using the Mycoplasma IST kit (BioMerieux, Marcy L' Etoile, France). For any other microorganism, we applied general culture.

6.3.1.2. Clinical assessment

An infertility medical history was taken and recorded on a standardized form by a single experienced clinician, who also performed external genital, vaginal exploration, pelvic examinations and realized the sampling of genital secretions. The data obtained from medical file were: demographic parameters, history of fertility and infertility, gestational losses and obstetric complications, treatment of infertility problems, history of cervical lesions, and surgical procedures, result of previous cervical-vaginal cytology Pap smears, signs and symptoms of genitourinary infections and pathological data of the cervix and vagina examination. At subjective appreciation, the clinical parameters were considered for semiquantitative record: low, medium, and high or abundant.

6.3.1.3. HPV DNA extraction and genotyping

For the extraction and purification of DNA from cervical samples, 100 μL of the cell suspension previously prepared was used. Silica extraction columns (Nucleospin® Blood, Machery-Nagel GmBH & Co., Germany) were used under the manufacturer's instructions. During the 12 years covered by this study, the technology for HPV diagnosis was changing. For this reason, different techniques were applied depending on the time when the patient arrives for medical treatment. In 34 patients, molecular detection of HPV-AND was carried out by PCR-Multiplex that detect 4–12 genotypes; for 36 patients, secretions from the cervix and vagina were tested for HPV capillary electrophoresis using "Applied Biosystems 3130 series genetic analyzer systems" and the software GeneMapper, which diagnostic 14–19 genotypes high and low risk. Molecular detection of ADN from HPV was done in the 36 patients by

Dual Priming Oligonucleotide, more specific and that detect 14–19 genotypes of HPV and in the 34 last patients *in situ* Hybridization with oligo specific probes to identified 20–28 HPV genotypes.

6.3.1.4. Statistical analysis

Absolute frequency of qualitative parameters is expressed in percentages. For quantitative variable was calculated media and, or median as well standard deviation. Sensitivity, specificity, positive and negative predictive values were calculated using (2 × 2) contingency tables. The association between clinical results and HPV and the association between other microorganisms present were calculated with Fisher's exact test. For all tests, the significance level was $P \leq 0.05$ and power of $(1\text{-}\beta) = 80\%$ for two-tail. The statistical software used was SPSS version 17 for Windows (SPSS Inc.)

6.3.2. Results

From 690 infertile couples attended from 2003 to 2012, the aleatory selected sample of 104 female partners was representative, according to medium probability ponderation of 48% for the different genotypess, that was estimated based in some reports [122]. A total of 104 female partners from couples with genitourinary infection were included in this study. Primary infertility was present in 65 couples and secondary infertility in 13 couples. The median age of the patients was 34 years (range, 22–55 years). The microorganisms identified on the group studied are *C. trachomatis* (86.5%), HPV (49%), *U. urealyticum* (47.11%), *M. Hominis* (35.57%), *E. coli* (20.2%), *C. Albicans* (17.3%) and *S. faecalis* (10.6%). In a high proportion of the vaginal samples, microbiota vaginal was reduced or absent in a (76.9%) and 86% have more than one pathogen germs. *C albicans* was present like solitary microorganism in 17.3% as well HPV was the only identified germ in one patient. Statistical analysis established positive correlation between HPV and *U. urealyticum* ($p = 0.05$) and decreased microbiota ($p = 0.001$).

The distribution of *C. trachomatis* was similar in the positive and negative to HPV group (**Figure 1**).

Between the clinical parameters taken into account for this investigation, some of them presented statistics correlation to the presence of HPV: the presence of more than one sexual partner ($p = 0.05$), genitourinary infection symptoms ($p = 0.01$), white mucous vaginal secretion fluxing from the uterine duct ($p = 0.05$), failure in assisted reproduction previously carried out ($p \leq 0.0001$), and previous HPV infection diagnosed by suspicious lesion or cytological exam ($p \leq 0.0001$) was in association to HPV (**Table 1**). For *Microbiota vaginalis*, the relationship to the presence of HPV was proportionally inversed.

Prevalent genotypes of HPV detected in the infertile patients studied group were; HPVar 6 (19.2%), HPVhr 18 (12.5%), HPVhr 16 (11.6%), HPVhr 58 (11.1%), HPVhr 52 (7.1%), HPVlr 11 (6.7%) and HPVhr 68 (7.5%). Thirteen different genotypes of PVH were detected in low frequency. These data are concentrated on **Table 2**. To consider the percentage of this frequency, it is necessary to remember that the results depend on the number of HPV search in each one of the three different tests applied that was not equal for all patients.

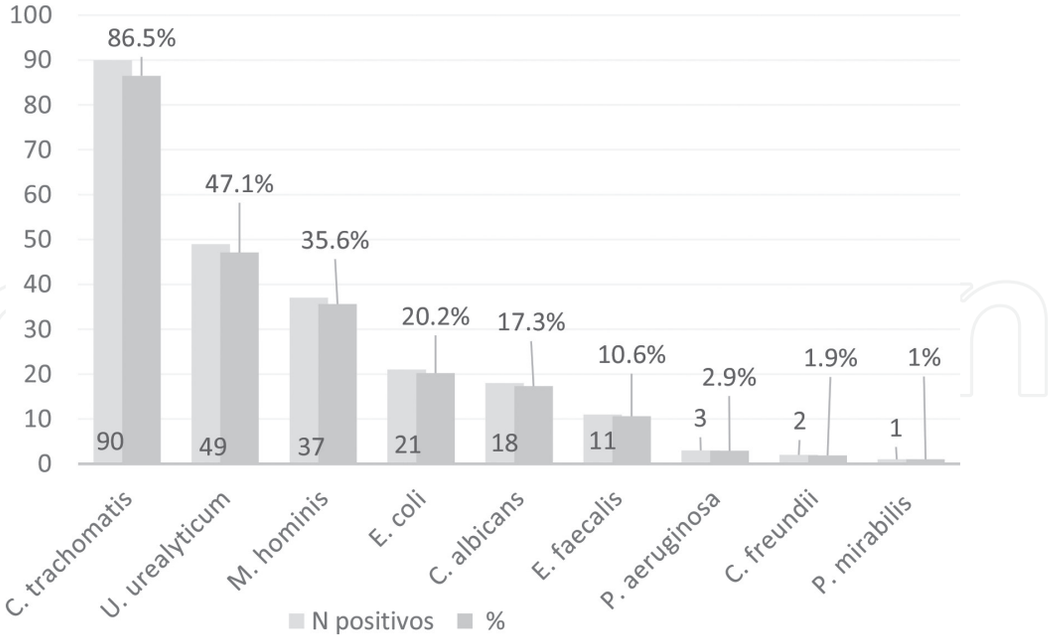

Figure 1. Microorganism identified into the cervix and vagina secretions of 104 female partners from couples with genitourinary infection. The most frequent bacteria in the genital sample of infertile women studied were *Chlamydia trachomatis*. However, if we considered that *Ureaplasma urealyticum* and *Mycoplasma hominis* belong to the same genus: Mycoplasmataceae, the presence of these bacteria, result of major importance in this group.

Combinations of more than one HPV genotypes were detected in 60% on the studied samples. From them 46.3% were the following associations between high risk for cancer HPV: 16/18, 18/58, 16/33, 16/52 and 18/52. In a similar percentage (44.8%) of women, low risk for cancer, principally HPV 6, was detected in combination with high risk HPV: 18, 52, 16 and 39. As well, we observed that three to five different HPV genotypes were present in 23.5% of the positive group infertile patients (**Figure 2**).

According to the results of this study, a total of 79.5% of positive HPV group of patients, presented one or more high-risk genotypes, and considering the statistical association between the positivity to HPV and the clinical history of previous HPV infections, this condition may represent a persistent infection or a reinfection. Consequently, we consider convenient a recommendation to take in account women with reproductive failure, like a group of risk of cervical cancer. There was statistical association between the positivity to HPV to clinical history of previous HPV infections. Would be very important, the simultaneously presence with *Ureaplasma urealyticum*, and *Chlamydia trachomatis*, sexually transmitted germs with suspicious carcinogenic potential, in a population in reproductive age, as we observed in this study.

With the results obtained from this study, it was not possible to establish an association between the abortions presented in 28 of the studied women, and the positivity to HPV. Nevertheless for future studies that include a high number of patients, is may be important to observe that low risk to cancer genotypes 6 and 11, were present in a high prevalence in this investigation, and their importance on the results of inseminations, in vitro fertilization and gestational loss, and are not defined now a days. Finally, our data confirm the recommendation to investigate HPV before to carry out *in vitro* fertilization, in view of the high frequency of failure of this treatment of infertility, when HPV is present.

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	Total of cases		HPV positive		HPV negative		P
	N	%	N	%	N	%	
HPV previous diagnosis	46	44.2	33	64.7	13	24.5	0.0001
Pap. report: inflammation low/ moderate	33	32.0	21	41.2	12	23.1	0.05
Active genitourinary infection	67	64.4	39	76.5	28	52.8	0.01
Only one partner	67	64.4	28	54.9	39	73.6	0.05
≥2 sexual partner	37	35.6	23	45.1	14	26.4	0.05
Unsuccessfully FIV/ ICSI	15	100	11	73.3	4	26.7	0.002
White, dense mucus secretion	78	85.7	42	85.7	36	69.2	0.05
Ureaplasma urealyticum in vaginal secretion	49	47.1	29	56.9	20	37.7	0.05
Absence of vaginal microbiota	55	52.9	24	47.1	31	58.5	0.03
Diminished vaginal microbiota	25	24.0	17	33.3	8	15.1	0.03

The most frequent bacteria in the genital sample of infertile women studied were *Chlamydia trachomatis*. However, if we considered that *Ureaplasma urealyticum* and *Mycoplasma hominis* belong to the same genus: Mycoplasmataceae, the presence of these bacteria, result of major importance in this group.

Table 1. Microbiological and clinical data associated with HPV.

HPV genotype identified	Risk for cancer	Patients studied for genotype		Positive result	
		N	%	N	%
6	Low	104	100	20	19.2
16	Alto	103	99	12	11.6
18	High	104	100	13	12.5
58	High	72	69.2	8	11.1
52	High	98	94.2	7	7.1
11	Low	104	100	7	6.7
31	High	87	83.7	5	5.7
33	High	72	69.2	5	6.9
68	High	67	64.4	5	7.5
66	High	67	64.4	3	4.5
51	High	98	94.2	3	3.1

HPV genotype identified	Risk for cancer	Patients studied for genotype		Positive result	
		N	%	N	%
42	Low	66	63.5	2	3
39	High	69	66.3	3	4.3
59	High	69	66.3	1	1.4
45	High	73	69.2	1	1.4
35	High	73	69.2	1	1.4
71	Low	70	67.3	1	1.4
61	Low	69	66.3	1	1.4
55	Low	63	60.6	1	1.6
81	Low	1	1	1	–

Table 2. Prevalent genotypes of HPV detected by PCR in secretions of cervix and vagina of infertile women studied group.

The following description will give an illustration of some of cases included in this study resume of microbiologic and gineco obstetric data are included at the **Figures 3–5**.

Case 2. Women 34 years, previously treated with five intrauterine inseminations and three *in vitro* fertilization (IVF) with intracytoplasmic sperm injection (ICSI). This patient had been treated repeatedly too, for genitourinary infection, unsuccessfully. After abnormal findings in a colposcopy, and histopathologic diagnosis of koilocyte in a cervix biopsy the clinician decide to practice hysterectomy, principally to solve the recurrent infections. One year later, these women required medical assistance due to severe signs and symptoms of genitourinary infection. In the vaginal sample were presented HPV 18, 35, 39, 51, 56, 66 y 74 (**Figure 3**).
Case 3. Woman of 29 years old attended by primary infertility of unknown etiology and had been treated two times by assisted reproduction, with negative results. At the time of her arrive to the specially clinic, she referred previous result of ASCUS in PAP and signs and symptoms of genitourinary infection: dysuria, pelvic pain, abnormal menstrual bleeding, and a fluxing of white secretion from the vagina. At genital exploration, hypertrophy and erythema of cervix and papillary lesion at vaginal wall were found. The secretion of cervix and vagina was positive to *C. trachomatis*, *U. urealyticum* and *C. albicans*. PCR detected genotypes 16, 31, 33, 52, and 54 (**Figure 4**).

Case 3. Female 31 years, with two pregnancy loss of the first trimester, in both were diagnosed by ultrasound a chorionic vesicle without embryo. This patient did not have any fertility treatment previously. She had some PAP inform with inflammation but in one of them was diagnosed metaplastic changes. Genital infection was clinically diagnosed, and multi-microbial infection was detected in samples of secretions. (**Figure 4**). **Case 4.** A 37-year-old woman, who had a child without fertility treatment and did not have a second pregnancy, despite having no contraceptive treatment. This patient required medical assistance for chronic infection that was resistant to many treatments implemented previously. In this case were found HPV 44

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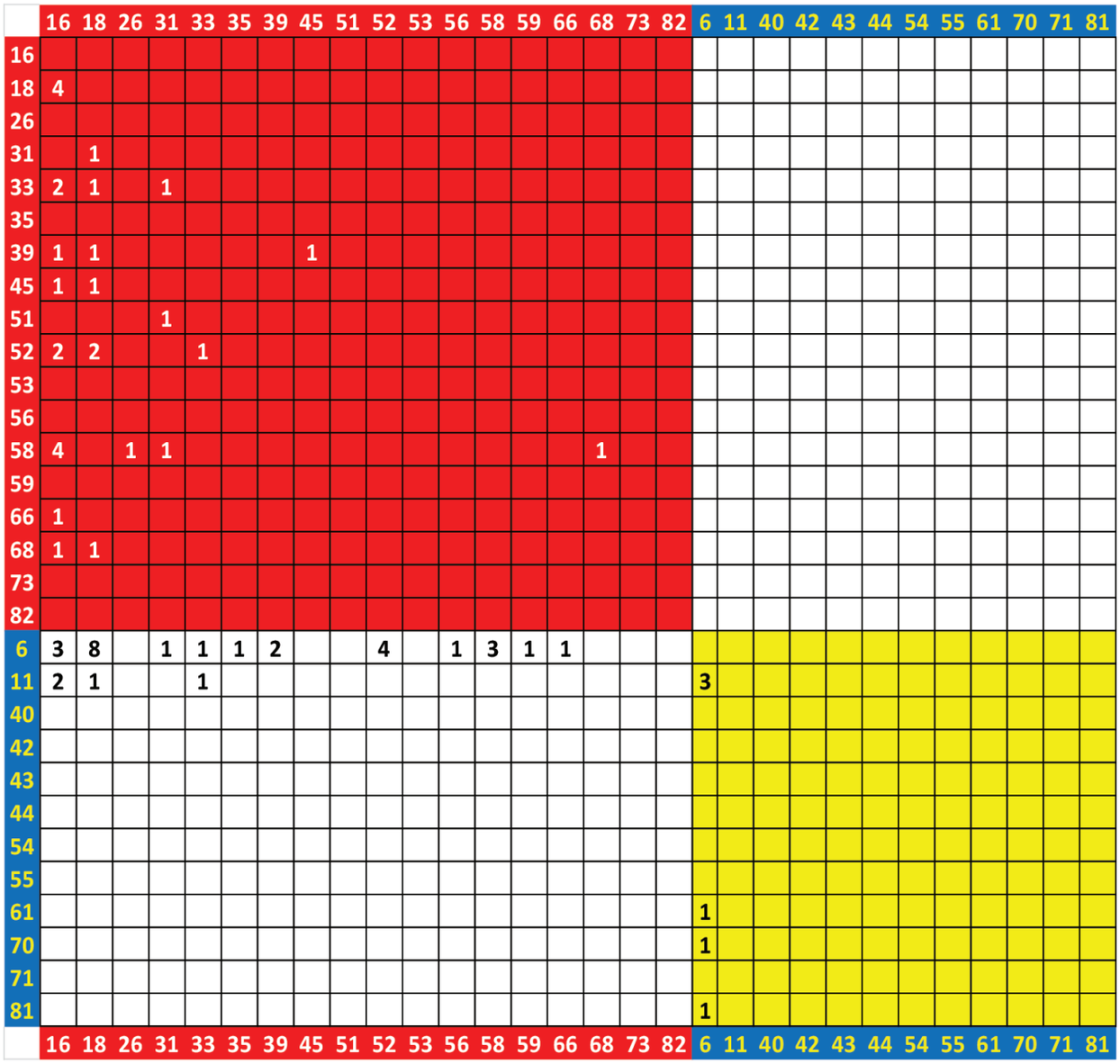

Figure 2. HPV genotyping results: Combinations of HPV. The number inside the square represents the patients with at least two different genotypes HPV detected in cervical and vaginal secretions of the women studied. Inside the square is the number of cases identified with the combined presence in the sample of cervical secretion of different Genotype HPV. For example: 4 cases have a combination of 16 and 18 genotypes of HPVhr; another 4 cases have simultaneously HPVhr 16 and 58.

and HPV 52 in association to *C. trachomatis*, *U. urealyticum* that eventually may be an etiologic association to secondary infertility of this case. Then more, *S. faecalis* and *E.coli* were present as part of the microorganism found in genital secretions. *Lactobacillus acidophilus* was absent (**Figure 5**). **Case 5**. Another patient that not received infertility treatment, but do not have a baby, even to be active sexually and do not have protection contraceptive. This woman suffers urinary symptoms since 2 years ago, and clinical signs and symptoms of vaginal recurrent infection, resistant to previous treatments. The diagnostic test applied to vaginal and cervical fluids was positive to one of the most common HPV associations: 16 and 18, but HPV 39, *C. trachomatis*, *U. urealyticum* and *C. albicans* were part of the multiple germs infection (**Figure 5**).

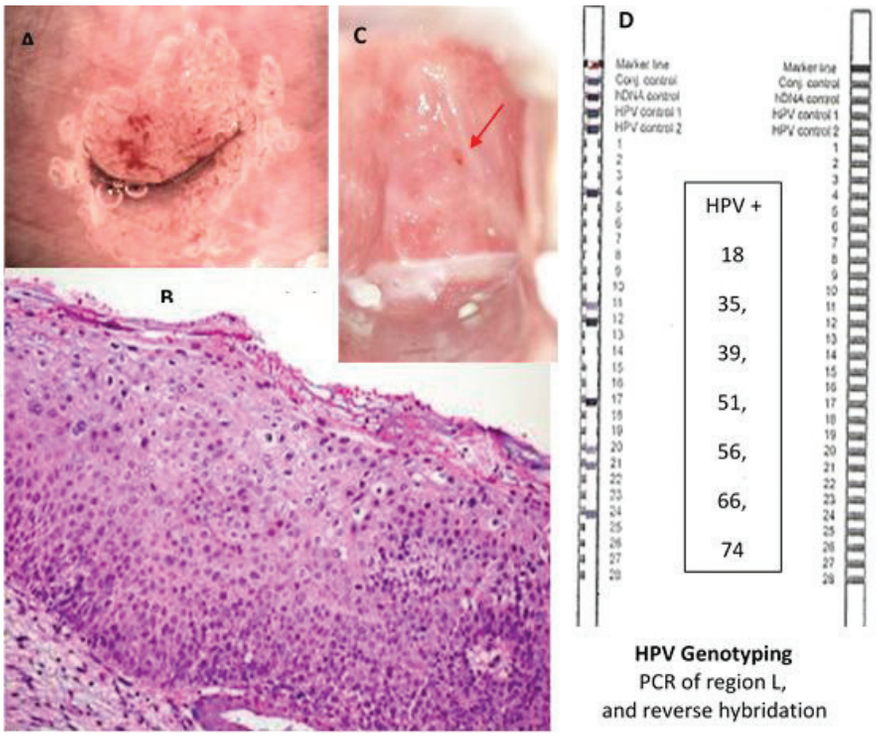

Figure 3. Infertile women with chronic genitourinary infection, and failure in assisted reproduction. Case 1. The patient was treated by hysterectomy due to persistent infection, after several fruitless treatment of assisted reproduction for primary infertility. The images illustrate clinical, histopathological and HPV test in vaginal secretions. (A) Abnormal colposcopy; (B) koilocyte was found in the cervix biopsy; (C) arrow shows lesion on vaginal fundus; (D) results of PCR and HPV genotyping of vaginal secretions. Multiple HPV were present, except the 74 of unknown significance, all correspond a high risk for cancer genotype.

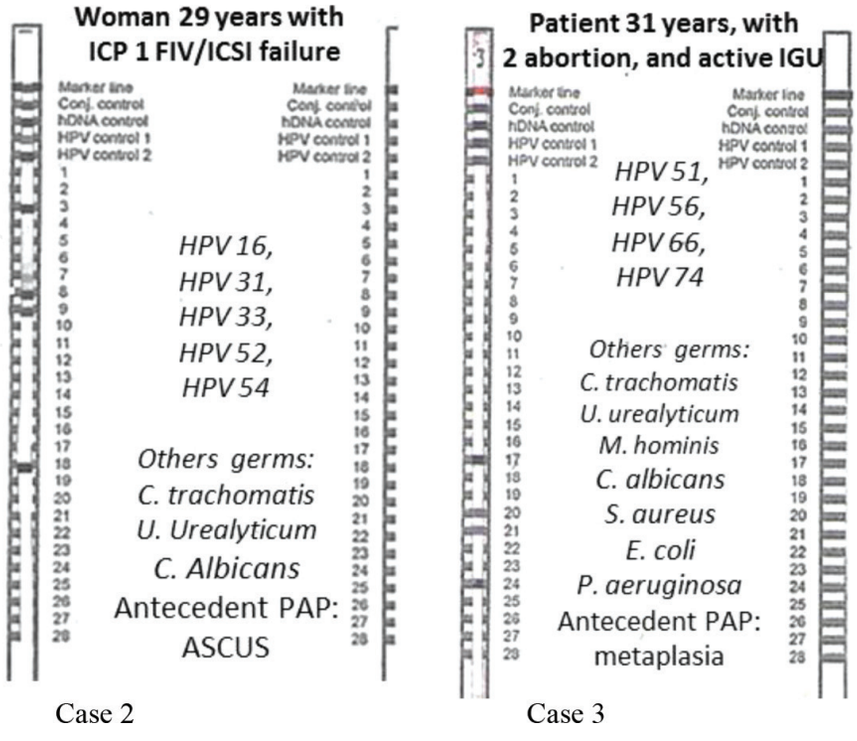

Figure 4. HPV genotypes combinations associated with genitourinary infection by multiple microorganisms.

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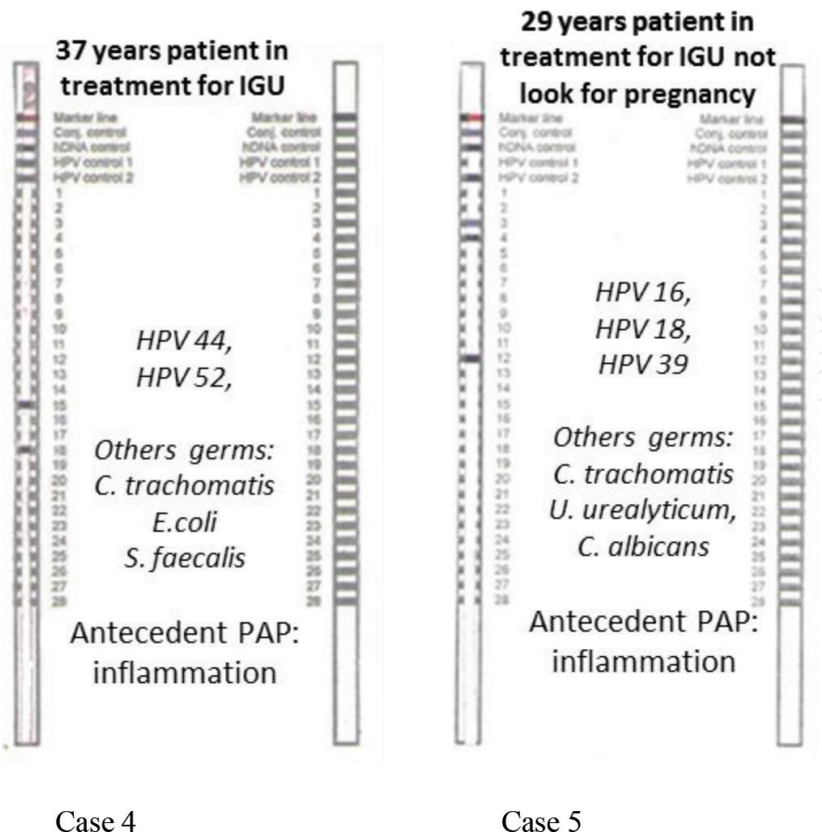

Figure 5. HPV genotypes combinations associated with genitourinary infection by multiple microorganisms in infertile patients.

7. Concluding remarks

This is a study carried out by aleatory selection of 104 infertile women attended by reproductive failure and genital infection; the main objective was to establish the relationship of HPV presence, with other microorganism and the genotype of HPV detected in this group of infertile women. All patients authorized the use of clinic and laboratory analysis data from her cervical and vaginal samples, to realize statistical analysis and sign an informed consent. The clinical parameters considered were: signs and symptoms of infection disease, results of previous assisted reproduction treatments, and the presence of previous abortions. These data were compared to genotype HPV present, and the diagnosis of others germs by laboratory test included direct investigation with immunofluorescence monoclonal antibodies to *Chlamydia trachomatis,* and bacterial cultures, included selective media to *U. urealyticum*, *M. hominis*. The microorganisms identified on the group studied are *C. trachomatis* (86.5%), HPV (49%), *U. urealyticum* (47.11%), *M. Hominis* (35.57%), *E. coli* (20.2%), *C. Albicans* (17.3%) and *S. faecalis* (10.6%). In a high proportion of the vaginal samples, microbiota vaginal was reduced or absent in a (76.9%) and 86% have more than one pathogen germs. *C albicans* was present like solitary microorganism in 17.3% as well HPV was the only identified germ in one patient. Statistical analysis established positive correlation between HPV and *U. urealyticum* ($p = 0.05$), and decreased microbiota ($p = 0.001$). The distribution of *C. trachomatis* was similar in the positive and negative to HPV group.

In this study, more than one sexual partner ($p = 0.05$), genitourinary infection symptoms ($p = 0.01$), white mucous vaginal secretion fluxing from the uterine duct ($p = 0.05$), failure in assisted reproduction before carry out, and previous HPV infection diagnosed by suspicious lesion or cytological exam ($p \leq 0.0001$) were in association to HPV. Prevalent genotypes of HPV detected in the infertile patients studied group were HPV six (19.2%), HPV 18 (12.5%), HPV 16 (11.5%), HPV 58 (7.2%), HPV 52 (6.7%), HPV 11 (6.7%) and HPV 68 (7.5%). Combinations of more than one HPV genotypes were detected in 60% on the studied samples. From them, 46.3% were the following associations between high risk for cancer HPV: 16/18, 18/58, 16/33, 16/52 and 18/52. In a similar percentage (44.8%) of women, low risk for cancer, principally HPV 6, was detected in combination with high risk HPV: 18, 52, 16 and 39. As well, we observed that three to five different HPV genotypes were present in 23.5% of the positive group infertile patients. According to the results of this study, a total of 79.5% of positive HPV group of patients, presented one or more high risk genotypes, and considering the statistical association between the positivity to HPV and the clinical history of previous HPV infections, this condition may represent a persistent infection or a reinfection. Consequently, we consider convenient a recommendation to take in account women with reproductive failure, like a group of risk of cervical cancer. With the results obtained from this study, was not possible to establish an association between the abortions presented on 28 of the studied women, and the positivity to HPV. Nevertheless for future studies that include a high number of patients, is may be important to observe that low risk to cancer genotypes 6 and 11, were present in a high prevalence in this investigation, and their importance on the results of inseminations, *in vitro* fertilization and gestational loss, still not defined now a days.

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